(19)日本国特許庁（ＪＰ）　(12)公開特許公報（Ａ）　(11)特許出願公開番号
特開2002－125657
（Ｐ２００２－１２５６５７Ａ）
(43)公開日　平成14年５月８日(2002.5.8)

(51)Int.Cl.[7]	識別記号	ＦＩ	テーマコード（参考）
C12N 1/20		C12N 1/20	A 4B024
			D 4B065
			F 4D040
C02F 3/34		C02F 3/34	Z 4D059
11/02		11/02	

審査請求　未請求　請求項の数４　ＯＬ　（全５頁）　最終頁に続く

(21)出願番号　特願2000－321025(Ｐ2000－321025)

(22)出願日　平成12年10月20日(2000.10.20)

(71)出願人　000005902
三井造船株式会社
東京都中央区築地５丁目６番４号
(71)出願人　395000555
入江　鎌三
長野県伊那市美篶7448－66
(72)発明者　高岡　一栄
千葉県市原市八幡海岸通１番地　三井造船株式会社千葉事業所内
(74)代理人　100076587
弁理士　川北　武長

最終頁に続く

(54)【発明の名称】バチルス・サブチリスに属する新規微生物

(57)【要約】　（修正有）
【課題】有機性廃液及び／又は生物性汚泥に含まれるタンパク質を分解することができるプロテアーゼ活性の高い性能を備えた新規微生物の提供。
【解決課題】(1) 有機性廃液及び／又は生物性汚泥に含まれるタンパク質を分解する性能を備えたバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。(2) 前記有機性廃液及び／又は生物性汚泥が下水余剰汚泥である前記記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。(3) 受託番号ＦＥＲＭ　Ｐ－１８０１５として寄託されている前記記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

1

【特許請求の範囲】

【請求項１】　有機性廃液および／または生物性汚泥に含まれるタンパク質を分解する性能を備えたバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

【請求項２】　前記有機性廃液および／または生物性汚泥が下水余剰汚泥である請求項１に記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

【請求項３】　受託番号ＦＥＲＭ　Ｐ－１８０１５として寄託されている請求項１または２に記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

【請求項４】　前記新規微生物が下記Ａ～Ｄの菌学的性質を有することを特徴とする請求項１～３のいずれかに記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

Ａ．形態的性質　(1) 細胞の形：桿菌、(2) 運動性の有無：＋、(3) 胞子の有無：＋、(4) グラム染色：＋

Ｂ．培地における生育状態　(1) 標準寒天培養：＋

Ｃ．生理学的性質　(1) グラム染色性：＋、(2) 硝酸塩の還元能：＋、(3) 脱窒反応：＋、(4) ＶＰテスト：＋、(5) インドールの生成：＋、(6) デンプンの加水分解：＋、(7) 大豆油分解性：＋、(8) 無機窒素源の利用：＋、(9) オキシダーゼ：＋、(10)カタラーゼ：＋、(11)生育の範囲：温度１３～５０℃、(12)酸素に対する態度：好気性、(13)Ｏ－Ｆ試験：グルコース　－＋、(14)アンモニアの利用性：＋、(15)ＮａＨＳの分解性：＋

Ｄ．遺伝学的性質　(1) Ｇ＋Ｃ含量：４２．７モル％、(2) １６ＳリボゾームＲＮＡのゲノムＤＮＡ解析（５ベース～１５４０ベース）によるバチルス・サブチリスに対する相同性：９９．８４％、(3) 塩基配列：配列番号１を有する。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は新規微生物に関し、さらに詳しくは有機性廃液および／または生物性汚泥に含まれるタンパク質を分解することができるバチルス・サブチリス（Bacillus Subtilis)に属する新規微生物に関する。

【０００２】

【従来の技術】従来、活性汚泥による有機物分解は汚泥中に生息する微生物の集団でなされており、特に分解性の高い菌に着目してこれらの菌類を積極的に有機物の分解に利用した例はない。

【０００３】

【発明が解決しようとする課題】近年、汚泥を発生しない屎尿処理場などから排出される生物性汚泥から、プロテアーゼ活性を示すを多くの菌種が単離されており、これらの菌の利用により、廃水および有機性／生物性汚泥に含まれるタンパク質成分を分解し、汚泥の発生量を抑える効果が期待されている。本発明の課題は、有機性廃液および／または生物性汚泥に含まれるタンパク質を分解することができるプロテアーゼ活性の高い性能を備えた新規微生物を提供することにある。

【０００４】

【課題を解決するための手段】本発明者らは、有機性廃液および／または生物性汚泥中に含まれるタンパク質成分を効率的に分解する機能を備えた微生物を検索すべく鋭意検討を重ねた結果、所望の特性を有した細菌を単離し、その種まで同定するに到り、本発明を完成するに至ったものである。すなわち、本願で特許請求される発明は以下のとおりである。

【０００５】（１）有機性廃液および／または生物性汚泥に含まれるタンパク質を分解する性能を備えたバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

（２）前記有機性廃液および／または生物性汚泥が下水余剰汚泥である（１）に記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

（３）受託番号ＦＥＲＭ　Ｐ－１８０１５として寄託されている（１）または（２）に記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

【０００６】（４）前記新規微生物が下記Ａ～Ｄの菌学的性質を有することを特徴とする（１）～（３）のいずれかに記載のバチルス・サブチリスに属する新規微生物バチルス・サブチリスＸ－２。

Ａ．形態的性質　(1) 細胞の形：桿菌、(2) 運動性の有無：＋、(3) 胞子の有無：＋、(4) グラム染色：＋

Ｂ．培地における生育状態　(1) 標準寒天培養：＋

Ｃ．生理学的性質　(1) グラム染色性：＋、(2) 硝酸塩の還元能：＋、(3) 脱窒反応：＋、(4) ＶＰテスト：＋、(5) インドールの生成：＋、(6) デンプンの加水分解：＋、(7) 大豆油分解性：＋、(8) 無機窒素源の利用：＋、(9) オキシダーゼ：＋、(10)カタラーゼ：＋、(11)生育の範囲：温度１３～５０℃、(12)酸素に対する態度：好気性、(13)Ｏ－Ｆ試験：グルコース　－＋、(14)アンモニアの利用性：＋、(15)ＮａＨＳの分解性：＋

Ｄ．遺伝学的性質　(1) Ｇ＋Ｃ含量：４２．７モル％、(2) １６ＳリボゾームＲＮＡのゲノムＤＮＡ解析（５ベース～１５４０ベース）によるバチルス・サブチリスに対する相同性：９９．８４％、(3) 塩基配列：配列番号１を有する。

【０００７】本発明によって取得された新規微生物バチルス・サブチリスＸ－２の生物学的特徴を決定するために、この微生物が有する菌学的性質、すなわちＡ．形態的性質、Ｂ．培地における生育状態、Ｃ．生理学的性質およびＤ．遺伝学的性質に関して検定を行った。その結果を以下に示す。

【０００８】Ａ．形態的性質

(1) 細胞の形および大きさ：培地としてニュートリエントブロス（ＯｘｉｄＣＭ－１）０．８％、グルコース０．８％、乾燥酵母エキス０．０２％および食塩０．６％（いずれもＷ／Ｖ％）に寒天１．４Ｗ／Ｖ％を加えたものからなる寒天培地において３２℃で培養を行った。２４時間培養したところ、１．５×５μｍのグラム陽性の桿菌であり、さらに室温で５日間培養を行った場合、長い連鎖上のものが多く観察された。

【０００９】(2) 運動性の有無：運動性は懸濁標本で確認することができ、周毛性の運動であった。

(3) 胞子の有無：芽胞を形成し、形は卵円形で菌体より膨脹している。

(4) グラム染色：コロニー形成の初期のサンプリングで染色性を示した。この時点での細胞は栄養細胞であり、胞子化は認められなかった。

Ｂ．培地における生育状態 (1)標準寒天培地：３２℃２４時間培養のコロニーの形態は乳白色のしわのあるコロニーであった。

【００１０】Ｃ．生理学的性質

(1) グラム染色性：＋、(2) 硝酸塩の還元能：＋、(3) 脱窒反応：＋、(4) ＶＰテスト：＋、(5) インドールの生成：＋、(6) デンプンの加水分解：＋、(7)大豆油分解性：＋、(8) 無機窒素源の利用：＋、(9) オキシダーゼ：＋、(10)カタラーゼ：＋、

(11)生育の範囲：温度１３～５０℃

生育温度について、低温側は液体培地を用い、５～１５℃まで１℃刻みで設定し、２４時間での生育を観察した。高温側は４０、４５、５０、５５℃に設定し、液体培地と斜面培地で２４時間での生育を観察した。

(12)酸素に対する態度：好気性、(13)Ｏ－Ｆ試験：グルコース　－＋、(14)アンモニアの利用性：＋、(15)ＮａＨＳの分解性：＋

【００１１】Ｄ．遺伝学的性質

Ｇ＋Ｃ含量は４２．７モル％で、Bacillus subutilisの文献値(Holt J. G.Bergey's Manual of Systematic Bacteriology, 9th ed., Vol., ed. By P.H.A. Sneath, Williams$ Wilkins, Baltimore, 1986, pp.1104-1139）の範囲内であった。測定方法はあらかじめＭｇＳＯ$_4$・４～５H_2ＯとＭｎＳＯ$_4$を用いてそれぞれ１２００mg／ｌ溶液を調整し、滅菌後の培地に無菌的にMg^{+}として4mg／ｌ、Mn^{2+}として１．５mg／ｌとなるように加え、２７℃で４～５日間胞子形成直前まで培養を行った。遠心分離して得た菌体は０．１ＭＥＤＴＡ／０．１５ＭＮａＣｌで洗浄し、－２０℃保存した。一般的な方法に従ってＤＮＡを抽出した。ＤＮＡの分解はＤＮＡＧＣ分析キット（ヤマサ醤油社製）を用いて５０℃で１時間行った。Ｇ＋Ｃ含量の分析はＨＰＬＣ（島津製作所製ＬＣ－９ＡおよびＳＤＰＤ－６Ａ）を用い、２７０

ｎｍで検出し、カラムはＡＱ－３１２（（株）ワイエシイ製、６．０×１５０mm）を用い、１０mＭ　H_3ＰＯ$_4$－１０mＭ　H_2ＰＯ$_4$（ｐＨ３．５±０．０１）、流量１．８ml／ｍｉｎで溶出させた。

【００１２】遺伝学的解析方法として１６ＳリボゾームＲＮＡの解析を採用した。この解析は、一般に行われている方法で回収した菌のＤＮＡを、PE Applied Biosystems社製のキットを使用し、このプロトコールに従って１６ＳｒＲＮＡの増幅を行った。なお、使用したプライマーは５ｆ、３３８ｆ、５１５ｆ、７７６ｆ、１０８７ｆ、１１７４ｆおよび３５７ｒ、５３１ｒ、８１０ｒ、１１０４ｒ、１１９３ｒ、１５４０ｒである。ＰＣＲの温度条件は９５℃３０秒、６０℃３０秒、７２℃４５秒で３０ｃｙｃｌｅ行った。シークエンスはＡＢＩ　ＰＲＩＳＭ　３１０を使用して解析した。得られたシークエンスデータはPE Applied Biosystems 社のMicroSeq data baseを使用してバチルス・サブチリスに対する相同性を算出した。相同性は９９．８４％であった。解析した塩基配列は５ｂａｓｅから１５４０ｂａｓｅであり、配位番号１であった。

【００１３】上記した新規微生物の諸性質はバチルス・サブチリスが所有する諸性質とよく対応したのでバチルス・サブチリスＸ－２（Bacillus subtilis X-2)と命名した。なお、得られたバチルス・サブチリスＸ－２は、平成１２年９月４日に本願出願人によって茨城県つくば市東町１丁目１番３号に所在の通商産業省工業技術院生命工学工業技術研究所にて寄託され、受託番号ＦＥＲＭＰ－１８０１５が付与されている。本発明はこの寄託微生物自体はもちろん、前述した能力を有するその変異体および子孫をも含むものである。

【００１４】

【発明の実施の形態】本発明における新規微生物バチルス・サブチリスＸ－２は以下のようにして単離した。採取した汚泥をブレンダーで１０秒間分散した汚泥懸濁液０．１mlを滅菌した０．６％食塩水で10^2、10^4、10^6倍希釈した。各希釈液０．１mlを後述のＡ培地からなる寒天平面に撒き、３２℃で培養した。細菌の識別はコロニーの形状、菌体の顕微鏡観察および生化学試験によった。また、平面や斜面でＢａｃｉｌｌｕｓ属菌は胞子を形成するので、コロニーが出現してから２～５日後に各コロニー菌株の胞子形成の有無を判定した。複数の菌株がコロニー中で混じっているときはさらに希釈法で分離した。グラム陰性菌とＢａｃｉｌｌｕｓ属菌が希釈法で分離できなかったときは、下記のＡ培地からなる斜面で培養して生じたＢａｃｉｌｌｕｓ属菌の胞子を滅菌した０．６％食塩水に懸濁し、８５℃１０分間加熱して分離できないグラム陰性菌を除いて単離した。

Ａ培地組成

ニュートリエントブロル（Ｏｘｏｉｄ　ＣＭ－１）　　８ｇ

5　　6

グルコース	８ｇ
ＮａＣｌ	６ｇ
乾燥酵母エキス（Ｄｉｆｃｏ）	０．２ｇ
寒天	１４ｇ
ＤＷ	１０００ｍｌ

【００１５】単離した３種の菌株Ｘ－１、Ｘ－２、Ｘ－１０について、タンパク質分解性の試験を行った。タンパク質分解試験は、クックドミート（日水製薬社製、ＯｃｉｄＣＭ－８１を使用）２００ｍｇを０．５％食塩水６ｍｌに懸濁し、３２℃８日間振とう培養して残留する懸濁物質量を計測し、懸濁物質消費率を下記式により求めることにより行った。

懸濁物消費率（％）＝［（Ａ－Ｂ）／Ａ］×１００

Ａ：加えたクックドミート懸濁物質量

Ｂ：残留した懸濁物質量

【００１６】クックドミートのタンパク質分解性試験結果を下記に示したが、菌株Ｘ－２の懸濁物質消費率が非常に高く、プロテアーゼ活性の高い菌株であることが判明した。この菌株Ｘ－２をバチルス・サブチリスＸ－２と命名した。バチルス・サブチリスＸ－２は配列番号１の１６ＳｒＲＮＡの核酸塩基配列を有していた。

菌株名	懸濁物質消費率
Ｘ－２	８１％
Ｘ－１０	１９％
Ｘ－Ｉ	３％

【００１７】

【発明の効果】本発明の新規微生物バチルス・サブチリスＸ－２によれば、プロテアーゼ活性の高い性能を備えているため、有機性廃液および／または生物性汚泥に含まれるタンパク質を分解し、汚泥の発生量を大幅に低減することができる。

【００１８】

【配列表】

<160> 1
<210> 1
<211> 1535
<212> DNA
<213> Bacillus subtilis
<400> 1
tgatcctggc tcaggacgaa cgctggcggc gtgcctaata catgcaagtc gagcggacag 60
atgggaagct tgctccctga tgtaagcggc ggacgggtga gtaacacgtg ggtaacctgc 120
ctgtaagact gggataactc cgggaaaccg gggctaatac cggatggttg tttgaaccgc 180
atggttcaaa cataaaaggt ggcttcggct accacttaca gatggacccg cggcgcatta 240
gctagttggt gaggtaacgg ctcaccaagg caacgatgcg tagccgacct gagagggtga 300
tcggccacac tgggactgag acacggccca gactcctacg ggaggcagca gtagggaatc 360
ttccgcaatg gacgaaagtc tgacggagca acgccgcgtg agtgatgaag gttttcggat 420
cgtaaagctc tgttgttagg gaagaacaag taccgttcga atagggcggt accttgacgg 480
tacctaacca gaaagccacg gctaactacg tgccagcagc cgcggtaata cgtaggtggc 540
aagcgttgtc cggaattatt gggcgtaaag ggctcgcagg cggtttctta agtctgatgt 600
gaaagccccc ggctcaaccg gggagggtca ttggaaactg gggaacttga gtgcagaaga 660
ggagagtgga attccacgtg tagcggtgaa atgcgtagag atgtggagga acaccagtgg 720
cgaaggcgac tctctggtct gtaactgacg ctgaggagcg aaagcgtggg gagcgaacag 780
gattagatac cctggtagtc cacgccgtaa acgatgagtg ctaagtgtta gggggtttcc 840
gccccttagt gctgcagcta acgcattaag cactccgcct ggggagtacg gtcgcaagac 900
tgaaactcaa aggaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960
agcaacgcga agaaccttac caggtcttga catcctctga caatcctaga gataggacgt 1020
ccccttcggg ggcagagtga caggtggtgc atggttgtcg tcagctcgtg tcgtgagatg 1080
ttgggttaag tcccgcaacg agcgcaaccc ttgatcttag ttgccagcat tcagttgggc 1140
actctaaggt gactgccggt gacaaaccgg aggaaggtgg ggatgacgtc aaatcatcat 1200
gccccttatg acctgggcta cacacgtgct acaatggaca gaacaaaggg cagcgaaacc 1260
gcgaggttaa gccaatccca caaatctgtt ctcagttcgg atcgcagtct gcaactcgac 1320
tgcgtgaagc tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa tacgttcccg 1380

7　　8

ggcctigtac acaccgcccg tcacaccacg agagtttgta acacccgaag tcggtgaggt 1440
aacctttiag gagccagccg ccgaaggtgg gacagatgat tggggtgaag tcgtaacaag 1500
gtagccgtat cggaaggtgc ggctggatca cctcc 1535

フロントページの続き

(51)Int.Cl.⁷	識別記号	FI		テーマコード（参考）
// Ｃ１２Ｎ	1/00	Ｃ１２Ｎ	1/00	Ｓ
	15/09 ＺＮＡ	(Ｃ１２Ｎ	1/20	Ａ
(Ｃ１２Ｎ	1/20	Ｃ１２Ｒ	1:125)	
Ｃ１２Ｒ	1:125)	(Ｃ１２Ｎ	1/00	Ｓ
(Ｃ１２Ｎ	1/00	Ｃ１２Ｒ	1:125)	
Ｃ１２Ｒ	1:125)	Ｃ１２Ｎ	15/00 ＺＮＡＡ	

(72)発明者　中谷　龍男
千葉県市原市八幡海岸通１番地　三井造船株式会社千葉事業所内
(72)発明者　入江　鐮三
長野県伊那市美篶7448－66

Ｆターム(参考)　4B024 AA17 CA01 CA11 DA07 HA12
4B065 AA19X AC12 BA22 BB19 CA55
4D040 DD01 DD22
4D059 AA05 BA22

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-125657
(43)Date of publication of application : 08.05.2002

(51)Int.Cl. C12N 1/20
C02F 3/34
C02F 11/02
// C12N 1/00
C12N 15/09
(C12N 1/20
C12R 1:125)
(C12N 1/00
C12R 1:125)

(21)Application number : 2000-321025
(22)Date of filing : 20.10.2000

(71)Applicant : MITSUI ENG & SHIPBUILD CO LTD
IRIE RIYOUZO
(72)Inventor : TAKAOKA KAZUE
NAKATANI TATSUO
IRIE RIYOUZO

(54) NEW MICROORGANISM BELONGING TO BACILLUS SUBTILIS

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a new microorganism capable of decomposing protein contained in organic waste fluid and/or biological sludge, and therefore having protease activity of high capability.
SOLUTION: This new microorganism Bacillus Subtilis X-2 belonging to Bacillus Subtilis has capability of decomposing the protein contained in the organic waste fluid and/or the biological sludge. The organic waste fluid and/or the biological sludge preferably comprise sewage surplus sludge. The microorganism preferably comprises the Bacillus Subtilis X-2 which is deposited to be given an assignment number of FERM P-18015.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

CLAIMS

[Claim(s)]
[Claim 1] New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis equipped with the engine performance which disassembles the protein contained in organic nature waste fluid and/or living thing nature sludge.
[Claim 2] New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis according to claim 1 said organic nature waste fluid and/or whose living thing nature sludge are sewage excess sludge.
[Claim 3] Trust number FERM New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis according to claim 1 or 2 deposited as P-18015.
[Claim 4] New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis according to claim 1 to 3 characterized by said new microorganism having the mycology-property of following A-D.
A. Gestalt-property (1) The form of a cell: A Bacillus and (2) Motile existence : [+,] (3) Existence of a spore: +(4) Gram's stain: Growth condition in a +B. culture medium (1) Standard agar culture: +C. physiological property (1) Gram's stain nature : [+,] (2) Reduction ability of a nitrate: +(3) Denitrification reaction: +(4) VP test : [+,] (5) Generation of Indore: +(6) Hydrolysis of starch : [+,] (7) Soybean-oil resolvability: +(8) Use of the source of inorganic nitrogen: +(9) Oxidase : [+,] (10) -- catalase: -- the range:temperature of 13-50 degrees C of + and (11) growth (12) The attitude:aerotropism, the (13) O-F trial:glucose to oxygen - +, (14) -- availability [of ammonia]: -- resolvability:+D. genetic property of + and (15) NaHS (1) G+C content: -- 42.7-mol % -- (2) Genomic DNA analysis of 16S ribosomal RNA (the 5 base - 1540 base) Homology to the bacillus subtilis to depend: 99.84% and (3) Base sequence: It has the array number 1.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the new microorganism belonging to the bacillus subtilis (Bacillus Subtilis) which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge in more detail about a new microorganism.
[0002]
[Description of the Prior Art] Conventionally, the organic substance decomposition by active sludge is made in the ensemble of the microorganism which lives into sludge, and there is no example which used these funguses for disassembly of the organic substance positively paying attention to the high bacillus of especially resolvability.
[0003]
[Problem(s) to be Solved by the Invention] In recent years, from the living thing nature sludge discharged from the night soil treatment plant which does not generate sludge, many strains are isolated [activity / protease] in ****, the protein component contained in waste water, and organic nature / living thing nature sludge by use of these bacilli is decomposed, and the effectiveness of stopping the yield of sludge is expected. The technical problem of this invention is to offer the new microorganism equipped with the engine performance in which the protease activity which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge is high.
[0004]
[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the microorganism equipped with the function which decomposes efficiently the protein component contained in organic nature waste fluid and/or living thing nature sludge should be searched, this invention persons isolate bacteria with a desired property, come to identify even the kind, and come to complete this invention. That is, the invention by which a patent claim is carried out by this application is as follows.
[0005] (1) New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis equipped with the engine performance which disassembles the protein contained in organic nature waste fluid and/or living thing nature sludge.
(2) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) said organic nature waste fluid and/or whose living thing nature sludge are sewage excess sludge.
(3) Trust number FERM New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) deposited as P-18015, or (2).
[0006] (4) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in either of (1) - (3) characterized by said new microorganism having the mycology-property of following A-D.
A. Gestalt-property (1) The form of a cell: A Bacillus and (2) Motile existence : [+,] (3) Existence of a spore: +(4) Gram's stain: Growth condition in a +B. culture medium (1) Standard agar culture: +C. physiological property (1) Gram's stain nature : [+,] (2) Reduction ability of a nitrate: +(3) Denitrification reaction: +(4) VP test : [+,] (5) Generation of Indore: +(6) Hydrolysis of starch : [+,] (7) Soybean-oil resolvability: +(8) Use of the source of inorganic nitrogen: +(9) Oxidase : [+,] (10) -- catalase: -- the range:temperature of 13-50 degrees C of + and (11) growth (12) The attitude:aerotropism, the (13) O-F trial:glucose to oxygen - +, (14) -- availability [of ammonia]: -- resolvability:+D. genetic property of + and (15) NaHS (1) G+C content: -- 42.7-mol % -- (2) Genomic DNA analysis of 16S ribosomal RNA (the 5 base - 1540 base) Homology to the bacillus subtilis to depend: 99.84% and (3) Base sequence: It has the array number 1.
[0007] In order to determine the biological description of the new microorganism bacillus subtilis X-2 acquired by this invention, it authorized about the mycology-property which this microorganism has, i.e., A. gestalt-property, the growth condition in B. culture medium, the C. physiological property, and the D. genetic property. The result is shown below.
[0008] A. Gestalt-property (1) A form and magnitude of a cell: In the agar medium which consists of what added agar 1.4 W/V% to dry-yeast extractives 0.02% and 0.6% (all are W/V %) of salt, it cultivated at 32 degrees C glucose 0.8% neutrient broth (OxidCM-1) 0.8% as a culture medium. When cultivated for 24 hours, it was a 1.5x5-micrometer gram-positive Bacillus, and when culture was further performed for five days at a room temperature, many things on a long chain were observed.
[0009] (2) Motile existence : maneuverability could be checked by the suspension sample and was peritrichate movement.
(3) Existence of a spore : forming a spore, the form is expanding from the fungus body by the egg round shape.
(4) Gram's stain : the sampling in early stages of colony formation showed the dye affinity. The cell in this time is a vegetative cell, and spore-ization was not accepted.
B. growth condition in a culture medium (1) standard agar-medium: -- the gestalt of the colony of 32-degree-C 24-hour culture -- milk -- it was a colony with a white wrinkling.
[0010] C. Physiological property (1) Gram's-stain nature: +(2) Reduction ability of a nitrate : [+,] (3) Denitrification reaction: + (4) VP test: +(5) Generation of Indore : [+,] (6) Hydrolysis of starch: +, (7) soybean-oil resolvability:+, and (8) Use of the source of inorganic nitrogen : [+,] (9) oxidase: -- about the range:temperature 13 - 50-degree-C growth temperature of +, (10) catalase:+, and (11) growth, using the liquid medium, 1 degree C was minced, it came out, and the low temperature side was set up to 5-15 degrees C, and observed growth in 24 hours. The elevated-temperature side was set as 40, 45, and 50 or 55 degrees C, and observed growth in 24 hours by the liquid medium and the slant medium.
(12) The attitude:aerotropism, (13) O-F trials to oxygen : glucose Availability:+ of -+(14) ammonia, resolvability:[of (15) NaHS]+
[0011] D. A genetic property G+C content is 42.7-mol %, and is the reference value of Bacillus subutilis (Holt J.G.Bergey's Manual of Systematic Bacteriology, 9th ed., Vol., and ed.By P.H.A.Sneath, Williams$ Wilkins, Baltimore, 1986, and pp.1104-1139). It was within the limits. a measuring method -- beforehand -- MgSO4 and 4-5H2 -- O and MnSO4 it uses, and 1200 mg/l solutions are adjusted, respectively, and sterile to the culture medium after sterilization ---like -- Mg+ ****** -- in addition, it cultivated for four - five days at 27 degrees C just before sporulation so that it might become 1.5 mg/l as 4 mg/l and Mn2+. The obtained fungus body which carried out centrifugal separation was washed by 0.1MEDTA(s)/0.15MNaCl, and was saved -20 degrees C. DNA was extracted according to the general approach. Decomposition of DNA is DNA. It carried out at 50 degrees C for 1 hour using GC analysis kit (YAMASA soy sauce company make). Analysis of a G+C content is detected by 270nm using HPLC (Shimadzu LC-9A and SDPD-6A), a column uses AQ-312 (Product made from Wye ESHII, 6.0x150mm), and it is 10mM. H3 PO4-10mM Elution was carried out by H2 PO4 (pH 3.5**0.01) and flow rate 1.8 ml/min.
[0012] The analysis of 16S ribosomal RNA was adopted as the genetic analysis approach. This analysis used the kit made from

PE Applied Biosystems for DNA of the bacillus collected by the approach currently generally performed, and amplified 16SrRNA (s) according to this protocol. In addition, the used primers are 5f, 338f, 515f, 776f, 1087f, 1174f, and 357r, 531r, 810r, 1104r, 1193r and 1540r. The temperature conditions of PCR were performed 30 cycles in 72-degree-C 45 seconds for 60-degree-C 30 seconds for 95-degree-C 30 seconds. Sequence is ABI. PRISM It analyzed using 310. The obtained sequence data are PE Applied Biosystems. The homology to bacillus subtilis was computed using MicroSeq data base of a shrine. Homology was 99.84%. The analyzed base sequences were 1540base(s) from 5base(s), and were the coordination number 1.

[0013] Since many properties of the above-mentioned new microorganism corresponded well with many properties which bacillus subtilis owns, they were named the bacillus subtilis X-2 (Bacillus subtilis X-2). In addition, an applicant for this patent ****s to 1-1-3, **, Higashi, Tsukuba-shi, Ibaraki-ken in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the whereabouts, the Ministry of International Trade and Industry, on September 4, Heisei 12, and the obtained bacillus subtilis X-2 is the trust number FERM. P-18015 is given. This invention also contains that variant and descendant that have the capacity mentioned above as well as this deposition microorganism itself.

[0014]

[Embodiment of the Invention] The new microorganism bacillus subtilis X-2 in this invention is the following, and was made and isolated. It is 102, 104, and 106 with 0.6% brine which sterilized 0.1ml of sludge suspension which distributed the extracted sludge for 10 seconds with the blender. It double-diluted. 0.1ml of each diluent was scattered at the agar flat surface which consists of the below-mentioned A culture medium, and it cultivated at 32 degrees C. Bacterial discernment was based on microscope observation and biochemical study of the configuration of a colony, and a fungus body. Moreover, since Bacillus **** formed the spore on the flat surface or the slant face, after the colony appeared, the existence of the sporulation of each colony strain was judged two - five days after. When two or more strain was mixed all over the colony, it dissociated with the dilution method further. When a gram negative and Bacillus **** were not able to dissociate with a dilution method, it suspended in 0.6% brine which sterilized the spore of Bacillus **** cultivated and produced on the slant face which consists of the following A culture medium, and isolated except for the gram negative which heats for 10 minutes and cannot be separated 85 degrees C.

A medium composition Neutrient BURORU (Oxoid CM-1) 8g A glucose 8g NaCl 6g Dry-yeast extractives (Difco) 0.2g Agar 14g DW 1000ml [0015] Proteolysis nature was examined about three sorts of isolated strain X-1, X-2, and X-10. The proteolysis trial suspended KUKKUDO meat (NISSUI PHARMACEUTICAL CO., LTD. make and OcidCM-81 are used) 200mg in 6ml of brine 0.5%, 32 degrees C for eight days, measured the suspended solid which carries out shaking culture and remains, and was performed by searching for suspended solid specific consumption by the following formula.

suspended-solid specific-consumption (%) =[(A-B) /A] x100A: The applied KUKKUDO meat suspended solid B: The suspended solid which remained [0016] Although the proteolysis sex-test result of a KUKKUDO meat was shown below, the suspended solid specific consumption of strain X-2 was very high, and it became clear that it was strain with high protease activity. This strain X-2 was named the bacillus subtilis X-2. The bacillus subtilis X-2 had the nucleic-acid base sequence of 16SrRNA(s) of the array number 1.

菌株名	懸濁物質消費率
X－2	81%
X－10	19%
X－I	3%

[0017]

[Effect of the Invention] According to the new microorganism bacillus subtilis X-2 of this invention, since it has the engine performance in which protease activity is high, the protein contained in organic nature waste fluid and/or living thing nature sludge can be disassembled, and the yield of sludge can be reduced sharply.

[0018]

[Layout Table]

<160> 1 <210> 1 <211> 1535<212>DNA <213> Bacillus subtilis <400> 1 tgatcctggc-tcaggacgaa cgctggcggc-gtgcctaata catgcaagtc gagcggacag 60 atgggaagct tgctccctga tgtaagcggc ggacgggtga gtaacacgtg ggtaacctgc 120 ctgtaagact gggataactc cgggaaaccg gggctaatac cggatggttg tttgaaccgc 180 atggttcaaa cataaaaggt ggcttcggct accacttaca gatggacccg cggcgcatta 240 gctagttggt gaggtaacgg ctcaccaagg caacgatgcg tagccgacct gagagggtga 300 tcggccacac tgggactgag acacggccca gactcctacg ggaggcagca gtagggaatc 360 ttccgcaatg gacgaaagtc tgacggagca acgccgcgtg agtgatgaag gttttcggat 420 cgtaaagctc tgttgttagg gaagaacaag taccgttcga atagggcggt accttgacgg 480 tacctaacca gaaagccacg gctaactacg tgccagcagc cgcggtaata cgtaggtggc 540 aagcgttgtc cggaattatt gggcgtaaag ggctcgcagg cggtttctta agtctgatgt 600 gaaagccccc ggctcaaccg gggagggtca ttggaaactg gggaacttga gtgcagaaga 660 ggagagtgga attccacgtg tagcggtgaa atgcgtagag atgtggagga acaccagtgg 720 cgaaggcgac tctctggtct gtaactgacg ctgaggagcg aaagcgtggg gagcgaacag 780 gattagatac cctggtagtc cacgccgtaa acgatgagtg ctaagtgtta gggggtttcc 840 gccccttagt gctgcagcta acgcattaag cactccgcct ggggagtacg gtcgcaagac 900 tgaaactcaa aggaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960 agcaacgcga agaaccttac caggtcttga catcctctga caatcctaga gataggacgt 1020 ccccttcggg ggcagagtga caggtggtgc atggttgtcg tcagctcgtg tcgtgagatg 1080 ttgggttaag tcccgcaacg agcgcaaccc ttgatcttag ttgccagcat tcagttgggc 1140 actctaaggt gactgccggt gacaaaccgg aggaaggtgg ggatgacgtc aaatcatcat 1200 gccccttatg acctgggcta cacacgtgct acaatggaca gaacaaaggg cagcgaaacc 1260 gcgaggttaa gccaatccca caaatctgtt ctcagttcgg atcgcagtct gcaactcgac 1320 tgcgtgaagc tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa tacgttcccg 1380 ggccttgtac acaccgcccg tcacaccacg agagtttgta acacccgaag tcggtgaggt 1440 aacctttag gagccagccg ccgaaggtgg gacagatgat tggggtgaag tcgtaacaag 1500 gtagccgtat cggaaggtgc ggctggatca cctcc 1535

[Translation done.]

TECHNICAL FIELD

[Field of the Invention] This invention relates to the new microorganism belonging to the bacillus subtilis (Bacillus Subtilis) which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge in more detail about a new microorganism.

[Translation done.]

PRIOR ART

[Description of the Prior Art] Conventionally, the organic substance decomposition by active sludge is made in the ensemble of the microorganism which lives into sludge, and there is no example which used these funguses for disassembly of the organic substance positively paying attention to the high bacillus of especially resolvability.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention] According to the new microorganism bacillus subtilis X-2 of this invention, since it has the engine performance in which protease activity is high, the protein contained in organic nature waste fluid and/or living thing nature sludge can be disassembled, and the yield of sludge can be reduced sharply.

[0018]

[Layout Table]

<160> 1 <210> 1 <211> 1535<212> DNA <213> Bacillus subtilis <400> 1 tgatcctggc-tcaggacgaa cgctggcggc-gtgcctaata catgcaagtc gagcggacag60 atgggaagct tgctccctga tgtaagcggc ggacgggtga gtaacacgtg ggtaacctgc 120ctgtaagact gggataactc cgggaaaccg gggctaatac cggatggttg tttgaaccgc 180atggttcaaa cataaaaggt ggcttcggct accacttaca gatggacccg cggcgcatta 240gctagttggt gaggtaacgg ctcaccaagg caacgatgcg tagccgacct gagagggtga 300tcggccacac tgggactgag acacggccca gactcctacg ggaggcagca gtagggaatc 360ttccgcaatg gacgaaagtc tgacggagca acgccgcgtg agtgatgaag gttttcggat 420cgtaaagctc tgttgttagg gaagaacaag taccgttcga atagggcggt accttgacgg 480tacctaacca gaaagccacg gctaactacg tgccagcagc cgcggtaata cgtaggtggc 540aagcgttgtc cggaattatt gggcgtaaag ggctcgcagg cggtttctta agtctgatgt 600gaaagccccc ggctcaaccg gggagggtca ttggaaactg gggaacttga gtgcagaaga 660ggagagtgga attccacgtg tagcggtgaa atgcgtagag atgtggagga acaccagtgg 720cgaaggcgac tctctggtct gtaactgacg ctgaggagcg aaagcgtggg gagcgaacag 780gattagatac cctggtagtc cacgccgtaa acgatgagtg ctaagtgtta gggggtttcc 840gccccttagt gctgcagcta acgcattaag cactccgcct ggggagtacg gtcgcaagac 900tgaaactcaa aggaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960agcaacgcga agaaccttac caggtcttga catcctctga caatcctaga gataggacgt 1020ccccttcggg ggcagagtga caggtggtgc atggttgtcg tcagctcgtg tcgtgagatg 1080ttgggttaag tcccgcaacg agcgcaaccc ttgatcttag ttgccagcat tcagttgggc 1140actctaaggt gactgccggt gacaaaccgg aggaaggtgg ggatgacgtc aaatcatcat 1200gccccttatg acctgggcta cacacgtgct acaatggaca gaacaaaggg cagcgaaacc 1260gcgaggttaa gccaatccca caaatctgtt ctcagttcgg atcgcagtct gcaactcgac 1320tgcgtgaagc tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa tacgttcccg 1380ggccttgtac acaccgcccg tcacaccacg agagtttgta acacccgaag tcggtgaggt 1440aacctttta gagccagccg ccgaaggtgg gacagatgat tggggtgaag tcgtaacaag 1500 gtagccgtat cggaaggtgc ggctggatca cctcc 1535

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] In recent years, from the living thing nature sludge discharged from the night soil treatment plant which does not generate sludge, many strains are isolated [activity / protease] in ****, the protein component contained in waste water, and organic nature / living thing nature sludge by use of these bacilli is decomposed, and the effectiveness of stopping the yield of sludge is expected. The technical problem of this invention is to offer the new microorganism equipped with the engine performance in which the protease activity which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge is high.

[Translation done.]

MEANS

[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the microorganism equipped with the function which decomposes efficiently the protein component contained in organic nature waste fluid and/or living thing nature sludge should be searched, this invention persons isolate bacteria with a desired property, come to identify even the kind, and come to complete this invention. That is, the invention by which a patent claim is carried out by this application is as follows.
[0005] (1) New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis equipped with the engine performance which disassembles the protein contained in organic nature waste fluid and/or living thing nature sludge.
(2) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) said organic nature waste fluid and/or whose living thing nature sludge are sewage excess sludge.
(3) Trust number FERM New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) deposited as P-18015, or (2).
[0006] (4) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in either of (1) - (3) characterized by said new microorganism having the mycology-property of following A-D.
A. Gestalt-property (1) The form of a cell: A Bacillus and (2) Motile existence : [+,] (3) Existence of a spore: +(4) Gram's stain: Growth condition in a +B. culture medium (1) Standard agar culture: +C. physiological property (1) Gram's stain nature : [+,] (2) Reduction ability of a nitrate: +(3) Denitrification reaction: +(4) VP test : [+,] (5) Generation of Indore: +(6) Hydrolysis of starch : [+,] (7) Soybean-oil resolvability: +(8) Use of the source of inorganic nitrogen: +(9) Oxidase : [+,] (10) -- catalase: -- the range:temperature of 13-50 degrees C of + and (11) growth (12) The attitude:aerotropism, the (13) O-F trial:glucose to oxygen - +, (14) -- availability [of ammonia]: -- resolvability:+D. genetic property of + and (15) NaHS (1) G+C content: -- 42.7-mol % -- (2) Genomic DNA analysis of 16S ribosomal RNA (the 5 base - 1540 base) Homology to the bacillus subtilis to depend: 99.84% and (3) Base sequence: It has the array number 1.
[0007] In order to determine the biological description of the new microorganism bacillus subtilis X-2 acquired by this invention, it authorized about the mycology-property which this microorganism has, i.e., A. gestalt-property, the growth condition in B. culture medium, the C. physiological property, and the D. genetic property. The result is shown below.
[0008] A. Gestalt-property (1) A form and magnitude of a cell: In the agar medium which consists of what added agar 1.4 W/V% to dry-yeast extractives 0.02% and 0.6% (all are W/V %) of salt, it cultivated at 32 degrees C glucose 0.8% neutrient broth (OxidCM-1) 0.8% as a culture medium. When cultivated for 24 hours, it was a 1.5x5-micrometer gram-positive Bacillus, and when culture was further performed for five days at a room temperature, many things on a long chain were observed.
[0009] (2) Motile existence : maneuverability could be checked by the suspension sample and was peritrichate movement.
(3) Existence of a spore : forming a spore, the form is expanding from the fungus body by the egg round shape.
(4) Gram's stain : the sampling in early stages of colony formation showed the dye affinity. The cell in this time is a vegetative cell, and spore-ization was not accepted.
B. growth condition in a culture medium (1) standard agar-medium: -- the gestalt of the colony of 32-degree-C 24-hour culture -- milk -- it was a colony with a white wrinkling.
[0010] C. Physiological property (1) Gram's-stain nature: +(2) Reduction ability of a nitrate : [+,] (3) Denitrification reaction: + (4) VP test: +(5) Generation of Indore : [+,] (6) Hydrolysis of starch: +, (7) soybean-oil resolvability:+, and (8) Use of the source of inorganic nitrogen : [+,] (9) oxidase: -- about the range:temperature 13 - 50-degree-C growth temperature of +, (10) catalase:+, and (11) growth, using the liquid medium, 1 degree C was minced, it came out, and the low temperature side was set up to 5-15 degrees C, and observed growth in 24 hours. The elevated-temperature side was set as 40, 45, and 50 or 55 degrees C, and observed growth in 24 hours by the liquid medium and the slant medium.
(12) The attitude:aerotropism, (13) O-F trials to oxygen : glucose Availability:+ of -+(14) ammonia, resolvability:[of (15) NaHS]+
[0011] D. A genetic property G+C content is 42.7-mol %, and is the reference value of Bacillus subutilis (Holt J.G.Bergey's Manual of Systematic Bacteriology, 9th ed., Vol., and ed.By P.H.A.Sneath, Williams$ Wilkins, Baltimore, 1986, and pp.1104-1139). It was within the limits. a measuring method -- beforehand -- MgSO4 and 4-5H2 -- O and MnSO4 it uses, and 1200 mg/l solutions are adjusted, respectively, and sterile to the culture medium after sterilization ---like -- Mg+ ****** -- in addition, it cultivated for four - five days at 27 degrees C just before sporulation so that it might become 1.5 mg/l as 4 mg/l and Mn2+. The obtained fungus body which carried out centrifugal separation was washed by 0.1MEDTA(s)/0.15MNaCl, and was saved -20 degrees C. DNA was extracted according to the general approach. Decomposition of DNA is DNA. It carried out at 50 degrees C for 1 hour using GC analysis kit (YAMASA soy sauce company make). Analysis of a G+C content is detected by 270nm using HPLC (Shimadzu LC-9A and SDPD-6A), a column uses AQ-312 (Product made from Wye ESHII, 6.0x150mm), and it is 10mM. H3 PO4-10mM Elution was carried out by H2 PO4 (pH 3.5**0.01) and flow rate 1.8 ml/min.
[0012] The analysis of 16S ribosomal RNA was adopted as the genetic analysis approach. This analysis used the kit made from PE Applied Biosystems for DNA of the bacillus collected by the approach currently generally performed, and amplified 16SrRNA (s) according to this protocol. In addition, the used primers are 5f, 338f, 515f, 776f, 1087f, 1174f, and 357r, 531r, 810r, 1104r, 1193r and 1540r. The temperature conditions of PCR were performed 30 cycles in 72-degree-C 45 seconds for 60-degree-C 30 seconds for 95-degree-C 30 seconds. Sequence is ABI. PRISM It analyzed using 310. The obtained sequence data are PE Applied Biosystems. The homology to bacillus subtilis was computed using MicroSeq data base of a shrine. Homology was 99.84%. The analyzed base sequences were 1540base(s) from 5base(s), and were the coordination number 1.
[0013] Since many properties of the above-mentioned new microorganism corresponded well with many properties which bacillus subtilis owns, they were named the bacillus subtilis X-2 (Bacillus subtilis X-2). In addition, an applicant for this patent ****s to 1-1-3, **, Higashi, Tsukuba-shi, Ibaraki-ken in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the whereabouts, the Ministry of International Trade and Industry, on September 4, Heisei 12, and the obtained bacillus subtilis X-2 is the trust number FERM. P-18015 is given. This invention also contains that variant and descendant that have the capacity mentioned above as well as this deposition microorganism itself.
[0014]
[Embodiment of the Invention] The new microorganism bacillus subtilis X-2 in this invention is the following, and was made and isolated. It is 102, 104, and 106 with 0.6% brine which sterilized 0.1ml of sludge suspension which distributed the extracted sludge for 10 seconds with the blender. It double-diluted. 0.1ml of each diluent was scattered at the agar flat surface which consists of the below-mentioned A culture medium, and it cultivated at 32 degrees C. Bacterial discernment was based on

microscope observation and biochemical study of the configuration of a colony, and a fungus body. Moreover, since Bacillus **** formed the spore on the flat surface or the slant face, after the colony appeared, the existence of the sporulation of each colony strain was judged two - five days after. When two or more strain was mixed all over the colony, it dissociated with the dilution method further. When a gram negative and Bacillus **** were not able to dissociate with a dilution method, it suspended in 0.6% brine which sterilized the spore of Bacillus **** cultivated and produced on the slant face which consists of the following A culture medium, and isolated except for the gram negative which heats for 10 minutes and cannot be separated 85 degrees C.

A medium composition Neutrient BURORU (Oxoid CM-1) 8g A glucose 8g NaCl 6g Dry-yeast extractives (Difco) 0.2g Agar 14g DW 1000ml [0015] Proteolysis nature was examined about three sorts of isolated strain X-1, X-2, and X-10. The proteolysis trial suspended KUKKUDO meat (NISSUI PHARMACEUTICAL CO., LTD. make and OcidCM-81 are used) 200mg in 6ml of brine 0.5%, 32 degrees C for eight days, measured the suspended solid which carries out shaking culture and remains, and was performed by searching for suspended solid specific consumption by the following formula.

suspended-solid specific-consumption (%) =[(A-B) /A] x100A: The applied KUKKUDO meat suspended solid B: The suspended solid which remained [0016] Although the proteolysis sex-test result of a KUKKUDO meat was shown below, the suspended solid specific consumption of strain X-2 was very high, and it became clear that it was strain with high protease activity. This strain X-2 was named the bacillus subtilis X-2. The bacillus subtilis X-2 had the nucleic-acid base sequence of 16SrRNA(s) of the array number 1.

菌株名	懸濁物質消費率
X－2	８１％
X－１０	１９％
X－I	３％

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the new microorganism belonging to the bacillus subtilis (Bacillus Subtilis) which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge in more detail about a new microorganism.
[0002]
[Description of the Prior Art] Conventionally, the organic substance decomposition by active sludge is made in the ensemble of the microorganism which lives into sludge, and there is no example which used these funguses for disassembly of the organic substance positively paying attention to the high bacillus of especially resolvability.
[0003]
[Problem(s) to be Solved by the Invention] In recent years, from the living thing nature sludge discharged from the night soil treatment plant which does not generate sludge, many strains are isolated [activity / protease] in ****, the protein component contained in waste water, and organic nature / living thing nature sludge by use of these bacilli is decomposed, and the effectiveness of stopping the yield of sludge is expected. The technical problem of this invention is to offer the new microorganism equipped with the engine performance in which the protease activity which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge is high.
[0004]
[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the microorganism equipped with the function which decomposes efficiently the protein component contained in organic nature waste fluid and/or living thing nature sludge should be searched, this invention persons isolate bacteria with a desired property, come to identify even the kind, and come to complete this invention. That is, the invention by which a patent claim is carried out by this application is as follows.
[0005] (1) New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis equipped with the engine performance which disassembles the protein contained in organic nature waste fluid and/or living thing nature sludge.
(2) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) said organic nature waste fluid and/or whose living thing nature sludge are sewage excess sludge.
(3) Trust number FERM New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) deposited as P-18015, or (2).
[0006] (4) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in either of (1) - (3) characterized by said new microorganism having the mycology-property of following A-D.
A. Gestalt-property (1) The form of a cell: A Bacillus and (2) Motile existence : [+,] (3) Existence of a spore: +(4) Gram's stain: Growth condition in a +B. culture medium (1) Standard agar culture: +C. physiological property (1) Gram's stain nature : [+,] (2) Reduction ability of a nitrate: +(3) Denitrification reaction: +(4) VP test : [+,] (5) Generation of Indore: +(6) Hydrolysis of starch : [+,] (7) Soybean-oil resolvability: +(8) Use of the source of inorganic nitrogen: +(9) Oxidase : [+,] (10) -- catalase: -- the range:temperature of 13-50 degrees C of + and (11) growth (12) The attitude:aerotropism, the (13) O-F trial:glucose to oxygen - +, (14) -- availability [of ammonia]: -- resolvability:+D. genetic property of + and (15) NaHS (1) G+C content: -- 42.7-mol % -- (2) Genomic DNA analysis of 16S ribosomal RNA (the 5 base - 1540 base) Homology to the bacillus subtilis to depend: 99.84% and (3) Base sequence: It has the array number 1.
[0007] In order to determine the biological description of the new microorganism bacillus subtilis X-2 acquired by this invention, it authorized about the mycology-property which this microorganism has, i.e., A. gestalt-property, the growth condition in B. culture medium, the C. physiological property, and the D. genetic property. The result is shown below.
[0008] A. Gestalt-property (1) A form and magnitude of a cell: In the agar medium which consists of what added agar 1.4 W/V% to dry-yeast extractives 0.02% and 0.6% (all are W/V %) of salt, it cultivated at 32 degrees C glucose 0.8% neutrient broth (OxidCM-1) 0.8% as a culture medium. When cultivated for 24 hours, it was a 1.5x5-micrometer gram-positive Bacillus, and when culture was further performed for five days at a room temperature, many things on a long chain were observed.
[0009] (2) Motile existence : maneuverability could be checked by the suspension sample and was peritrichate movement.
(3) Existence of a spore : forming a spore, the form is expanding from the fungus body by the egg round shape.
(4) Gram's stain : the sampling in early stages of colony formation showed the dye affinity. The cell in this time is a vegetative cell, and spore-ization was not accepted.
B. growth condition in a culture medium (1) standard agar-medium: -- the gestalt of the colony of 32-degree-C 24-hour culture -- milk -- it was a colony with a white wrinkling.
[0010] C. Physiological property (1) Gram's-stain nature: +(2) Reduction ability of a nitrate : [+,] (3) Denitrification reaction: + (4) VP test: +(5) Generation of Indore : [+,] (6) Hydrolysis of starch: +, (7) soybean-oil resolvability:+, and (8) Use of the source of inorganic nitrogen : [+,] (9) oxidase: -- about the range:temperature 13 - 50-degree-C growth temperature of +, (10) catalase:+, and (11) growth, using the liquid medium, 1 degree C was minced, it came out, and the low temperature side was set up to 5-15 degrees C, and observed growth in 24 hours. The elevated-temperature side was set as 40, 45, and 50 or 55 degrees C, and observed growth in 24 hours by the liquid medium and the slant medium.
(12) The attitude:aerotropism, (13) O-F trials to oxygen : glucose Availability:+ of -+(14) ammonia, resolvability:[of (15) NaHS]+
[0011] D. A genetic property G+C content is 42.7-mol %, and is the reference value of Bacillus subutilis (Holt J.G.Bergey's Manual of Systematic Bacteriology, 9th ed., Vol., and ed.By P.H.A.Sneath, Williams$ Wilkins, Baltimore, 1986, and pp.1104-1139). It was within the limits. a measuring method -- beforehand -- MgSO4 and 4-5H2 -- O and MnSO4 it uses, and 1200 mg/l solutions are adjusted, respectively, and sterile to the culture medium after sterilization ---like -- Mg+ ****** -- in addition, it cultivated for four - five days at 27 degrees C just before sporulation so that it might become 1.5 mg/l as 4 mg/l and Mn2+. The obtained fungus body which carried out centrifugal separation was washed by 0.1MEDTA(s)/0.15MNaCl, and was saved -20 degrees C. DNA was extracted according to the general approach. Decomposition of DNA is DNA. It carried out at 50 degrees C for 1 hour using GC analysis kit (YAMASA soy sauce company make). Analysis of a G+C content is detected by 270nm using HPLC (Shimadzu LC-9A and SDPD-6A), a column uses AQ-312 (Product made from Wye ESHII, 6.0x150mm), and it is 10mM. H3 PO4-10mM Elution was carried out by H2 PO4 (pH 3.5**0.01) and flow rate 1.8 ml/min.
[0012] The analysis of 16S ribosomal RNA was adopted as the genetic analysis approach. This analysis used the kit made from

PE Applied Biosystems for DNA of the bacillus collected by the approach currently generally performed, and amplified 16SrRNA (s) according to this protocol. In addition, the used primers are 5f, 338f, 515f, 776f, 1087f, 1174f, and 357r, 531r, 810r, 1104r, 1193r and 1540r. The temperature conditions of PCR were performed 30 cycles in 72-degree-C 45 seconds for 60-degree-C 30 seconds for 95-degree-C 30 seconds. Sequence is ABI. PRISM It analyzed using 310. The obtained sequence data are PE Applied Biosystems. The homology to bacillus subtilis was computed using MicroSeq data base of a shrine. Homology was 99.84%. The analyzed base sequences were 1540base(s) from 5base(s), and were the coordination number 1.

[0013] Since many properties of the above-mentioned new microorganism corresponded well with many properties which bacillus subtilis owns, they were named the bacillus subtilis X-2 (Bacillus subtilis X-2). In addition, an applicant for this patent ****s to 1-1-3, **, Higashi, Tsukuba-shi, Ibaraki-ken in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the whereabouts, the Ministry of International Trade and Industry, on September 4, Heisei 12, and the obtained bacillus subtilis X-2 is the trust number FERM. P-18015 is given. This invention also contains that variant and descendant that have the capacity mentioned above as well as this deposition microorganism itself.

[0014]

[Embodiment of the Invention] The new microorganism bacillus subtilis X-2 in this invention is the following, and was made and isolated. It is 102, 104, and 106 with 0.6% brine which sterilized 0.1ml of sludge suspension which distributed the extracted sludge for 10 seconds with the blender. It double-diluted. 0.1ml of each diluent was scattered at the agar flat surface which consists of the below-mentioned A culture medium, and it cultivated at 32 degrees C. Bacterial discernment was based on microscope observation and biochemical study of the configuration of a colony, and a fungus body. Moreover, since Bacillus **** formed the spore on the flat surface or the slant face, after the colony appeared, the existence of the sporulation of each colony strain was judged two - five days after. When two or more strain was mixed all over the colony, it dissociated with the dilution method further. When a gram negative and Bacillus **** were not able to dissociate with a dilution method, it suspended in 0.6% brine which sterilized the spore of Bacillus **** cultivated and produced on the slant face which consists of the following A culture medium, and isolated except for the gram negative which heats for 10 minutes and cannot be separated 85 degrees C.

A medium composition Neutrient BURORU (Oxoid CM-1) 8g A glucose 8g NaCl 6g Dry-yeast extractives (Difco) 0.2g Agar 14g DW 1000ml [0015] Proteolysis nature was examined about three sorts of isolated strain X-1, X-2, and X-10. The proteolysis trial suspended KUKKUDO meat (NISSUI PHARMACEUTICAL CO., LTD. make and OcidCM-81 are used) 200mg in 6ml of brine 0.5%, 32 degrees C for eight days, measured the suspended solid which carries out shaking culture and remains, and was performed by searching for suspended solid specific consumption by the following formula.

suspended-solid specific-consumption (%) =[(A-B) /A] x100A: The applied KUKKUDO meat suspended solid B: The suspended solid which remained [0016] Although the proteolysis sex-test result of a KUKKUDO meat was shown below, the suspended solid specific consumption of strain X-2 was very high, and it became clear that it was strain with high protease activity. This strain X-2 was named the bacillus subtilis X-2. The bacillus subtilis X-2 had the nucleic-acid base sequence of 16SrRNA(s) of the array number 1.

菌株名	懸濁物質消費率
X－2	81%
X－10	19%
X－1	3%

[0017]

[Effect of the Invention] According to the new microorganism bacillus subtilis X-2 of this invention, since it has the engine performance in which protease activity is high, the protein contained in organic nature waste fluid and/or living thing nature sludge can be disassembled, and the yield of sludge can be reduced sharply.

[0018]

[Layout Table]

<160> 1 <210> 1 <211> 1535<212>DNA <213> Bacillus subtilis <400> 1 tgatcctggc-tcaggacgaa cgctggcggc-gtgcctaata catgcaagtc gagcggacag 60 atgggaagct tgctccctga tgtaagcggc ggacgggtga gtaacacgtg ggtaacctgc 120 ctgtaagact gggataactc cgggaaaccg gggctaatac cggatggttg tttgaaccgc 180 atggttcaaa cataaaaggt ggcttcggct accacttaca gatggacccg cggcgcatta 240 gctagttggt gaggtaacgg ctcaccaagg caacgatgcg tagccgacct gagagggtga 300 tcggccacac tgggactgag acacggccca gactcctacg ggaggcagca gtagggaatc 360 ttccgcaatg gacgaaagtc tgacggagca acgccgcgtg agtgatgaag gttttcggat 420 cgtaaagctc tgttgttagg gaagaacaag taccgttcga atagggcggt accttgacgg 480 tacctaacca gaaagccacg gctaactacg tgccagcagc cgcggtaata cgtaggtggc 540 aagcgttgtc cggaattatt gggcgtaaag ggctcgcagg cggtttctta agtctgatgt 600 gaaagccccc ggctcaaccg gggagggtca ttggaaactg gggaacttga gtgcagaaga 660 ggagagtgga attccacgtg tagcggtgaa atgcgtagag atgtggagga acaccagtgg 720 cgaaggcgac tctctggtct gtaactgacg ctgaggagcg aaagcgtggg gagcgaacag 780 gattagatac cctggtagtc cacgccgtaa acgatgagtg ctaagtgtta gggggtttcc 840 gcccccttagt gctgcagcta acgcattaag cactccgcct ggggagtacg gtcgcaagac 900 tgaaactcaa aggaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960 agcaacgcga agaaccttac caggtcttga catcctctga caatcctaga gataggacgt 1020 ccccttcggg ggcagagtga caggtggtgc atggttgtcg tcagctcgtg tcgtgagatg 1080 ttgggttaag tcccgcaacg agcgcaaccc ttgatcttag ttgccagcat tcagttgggc 1140 actctaaggt gactgccggt gacaaaccgg aggaaggtgg ggatgacgtc aaatcatcat 1200 gccccttatg acctgggcta cacacgtgct acaatggaca gaacaaaggg cagcgaaacc 1260 gcgaggttaa gccaatccca caaatctgtt ctcagttcgg atcgcagtct gcaactcgac 1320 tgcgtgaagc tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa tacgttcccg 1380 ggccttgtac acaccgcccg tcacaccacg agagtttgta acacccgaag tcggtgaggt 1440 aacctttta gagccagccg ccgaaggtgg gacagatgat tggggtgaag tcgtaacaag 1500 gtagccgtat cggaaggtgc ggctggatca cctcc 1535

[Translation done.]

TECHNICAL FIELD

[Field of the Invention] This invention relates to the new microorganism belonging to the bacillus subtilis (Bacillus Subtilis) which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge in more detail about a new microorganism.

[Translation done.]

PRIOR ART

[Description of the Prior Art] Conventionally, the organic substance decomposition by active sludge is made in the ensemble of the microorganism which lives into sludge, and there is no example which used these funguses for disassembly of the organic substance positively paying attention to the high bacillus of especially resolvability.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention] According to the new microorganism bacillus subtilis X-2 of this invention, since it has the engine performance in which protease activity is high, the protein contained in organic nature waste fluid and/or living thing nature sludge can be disassembled, and the yield of sludge can be reduced sharply.

[0018]

[Layout Table]

<160> 1 <210> 1 <211> 1535<212> DNA <213> Bacillus subtilis <400> 1 tgatcctggc-tcaggacgaa cgctggcggc-gtgcctaata catgcaagtc gagcggacag60 atgggaagct tgctccctga tgtaagcggc ggacgggtga gtaacacgtg ggtaacctgc 120ctgtaagact gggataactc cgggaaaccg gggctaatac cggatggttg tttgaaccgc 180atggttcaaa cataaaaggt ggcttcggct accacttaca gatggacccg cggcgcatta 240gctagttggt gaggtaacgg ctcaccaagg caacgatgcg tagccgacct gagagggtga 300tcggccacac tgggactgag acacggccca gactcctacg ggaggcagca gtagggaatc 360ttccgcaatg gacgaaagtc tgacggagca acgccgcgtg agtgatgaag gttttcggat 420cgtaaagctc tgttgttagg gaagaacaag taccgttcga atagggcggt accttgacgg 480tacctaacca gaaagccacg gctaactacg tgccagcagc cgcggtaata cgtaggtggc 540aagcgttgtc cggaattatt gggcgtaaag ggctcgcagg cggtttctta agtctgatgt 600gaaagccccc ggctcaaccg gggagggtca ttggaaactg gggaacttga gtgcagaaga 660ggagagtgga attccacgtg tagcggtgaa atgcgtagag atgtggagga acaccagtgg 720cgaaggcgac tctctggtct gtaactgacg ctgaggagcg aaagcgtggg gagcgaacag 780gattagatac cctggtagtc cacgccgtaa acgatgagtg ctaagtgtta ggggtttcc 840gccccttagt gctgcagcta acgcattaag cactccgcct ggggagtacg gtcgcaagac 900tgaaactcaa aggaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960agcaacgcga agaaccttac caggtcttga catcctctga caatcctaga gataggacgt 1020ccccttcggg ggcagagtga caggtggtgc atggttgtcg tcagctcgtg tcgtgagatg 1080ttgggttaag tcccgcaacg agcgcaaccc ttgatcttag ttgccagcat tcagttgggc 1140actctaaggt gactgccggt gacaaaccgg aggaaggtgg ggatgacgtc aaatcatcat 1200gccccttatg acctgggcta cacacgtgct acaatggaca gaacaaaggg cagcgaaacc 1260gcgaggttaa gccaatccca caaatctgtt ctcagttcgg atcgcagtct gcaactcgac 1320tgcgtgaagc tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa tacgttcccg 1380ggccttgtac acaccgcccg tcacaccacg agagtttgta acacccgaag tcggtgaggt 1440aacctttta gagccagccg ccgaaggtgg gacagatgat tggggtgaag tcgtaacaag 1500 gtagccgtat cggaaggtgc ggctggatca cctcc 1535

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] In recent years, from the living thing nature sludge discharged from the night soil treatment plant which does not generate sludge, many strains are isolated [activity / protease] in ****, the protein component contained in waste water, and organic nature / living thing nature sludge by use of these bacilli is decomposed, and the effectiveness of stopping the yield of sludge is expected. The technical problem of this invention is to offer the new microorganism equipped with the engine performance in which the protease activity which can disassemble the protein contained in organic nature waste fluid and/or living thing nature sludge is high.

[Translation done.]

MEANS

[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the microorganism equipped with the function which decomposes efficiently the protein component contained in organic nature waste fluid and/or living thing nature sludge should be searched, this invention persons isolate bacteria with a desired property, come to identify even the kind, and come to complete this invention. That is, the invention by which a patent claim is carried out by this application is as follows.
[0005] (1) New microorganism bacillus subtilis X-2 belonging to the bacillus subtilis equipped with the engine performance which disassembles the protein contained in organic nature waste fluid and/or living thing nature sludge.
(2) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) said organic nature waste fluid and/or whose living thing nature sludge are sewage excess sludge.
(3) Trust number FERM New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in (1) deposited as P-18015, or (2).
[0006] (4) New microorganism bacillus subtilis X-2 belonging to bacillus subtilis given in either of (1) - (3) characterized by said new microorganism having the mycology-property of following A-D.
A. Gestalt-property (1) The form of a cell: A Bacillus and (2) Motile existence : [+,] (3) Existence of a spore: +(4) Gram's stain: Growth condition in a +B. culture medium (1) Standard agar culture: +C. physiological property (1) Gram's stain nature : [+,] (2) Reduction ability of a nitrate: +(3) Denitrification reaction: +(4) VP test : [+,] (5) Generation of Indore: +(6) Hydrolysis of starch : [+,] (7) Soybean-oil resolvability: +(8) Use of the source of inorganic nitrogen: +(9) Oxidase : [+,] (10) — catalase: — the range:temperature of 13-50 degrees C of + and (11) growth (12) The attitude:aerotropism, the (13) O-F trial:glucose to oxygen - +, (14) — availability [of ammonia]: — resolvability:+D. genetic property of + and (15) NaHS (1) G+C content: — 42.7-mol % — (2) Genomic DNA analysis of 16S ribosomal RNA (the 5 base - 1540 base) Homology to the bacillus subtilis to depend: 99.84% and (3) Base sequence: It has the array number 1.
[0007] In order to determine the biological description of the new microorganism bacillus subtilis X-2 acquired by this invention, it authorized about the mycology-property which this microorganism has, i.e., A. gestalt-property, the growth condition in B. culture medium, the C. physiological property, and the D. genetic property. The result is shown below.
[0008] A. Gestalt-property (1) A form and magnitude of a cell: In the agar medium which consists of what added agar 1.4 W/V% to dry-yeast extractives 0.02% and 0.6% (all are W/V %) of salt, it cultivated at 32 degrees C glucose 0.8% neutrient broth (OxidCM-1) 0.8% as a culture medium. When cultivated for 24 hours, it was a 1.5x5-micrometer gram-positive Bacillus, and when culture was further performed for five days at a room temperature, many things on a long chain were observed.
[0009] (2) Motile existence : maneuverability could be checked by the suspension sample and was peritrichate movement.
(3) Existence of a spore : forming a spore, the form is expanding from the fungus body by the egg round shape.
(4) Gram's stain : the sampling in early stages of colony formation showed the dye affinity. The cell in this time is a vegetative cell, and spore-ization was not accepted.
B. growth condition in a culture medium (1) standard agar-medium: — the gestalt of the colony of 32-degree-C 24-hour culture — milk — it was a colony with a white wrinkling.
[0010] C. Physiological property (1) Gram's-stain nature: +(2) Reduction ability of a nitrate : [+,] (3) Denitrification reaction: + (4) VP test: +(5) Generation of Indore : [+,] (6) Hydrolysis of starch: +, (7) soybean-oil resolvability:+, and (8) Use of the source of inorganic nitrogen : [+,] (9) oxidase: — about the range:temperature 13 - 50-degree-C growth temperature of +, (10) catalase:+, and (11) growth, using the liquid medium, 1 degree C was minced, it came out, and the low temperature side was set up to 5-15 degrees C, and observed growth in 24 hours. The elevated-temperature side was set as 40, 45, and 50 or 55 degrees C, and observed growth in 24 hours by the liquid medium and the slant medium.
(12) The attitude:aerotropism, (13) O-F trials to oxygen : glucose Availability:+ of -+(14) ammonia, resolvability:[of (15) NaHS]+
[0011] D. A genetic property G+C content is 42.7-mol %, and is the reference value of Bacillus subutilis (Holt J.G.Bergey's Manual of Systematic Bacteriology, 9th ed., Vol., and ed.By P.H.A.Sneath, Williams$ Wilkins, Baltimore, 1986, and pp.1104-1139). It was within the limits. a measuring method — beforehand — MgSO4 and 4-5H2 — O and MnSO4 it uses, and 1200 mg/l solutions are adjusted, respectively, and sterile to the culture medium after sterilization ——like — Mg+ ****** — in addition, it cultivated for four - five days at 27 degrees C just before sporulation so that it might become 1.5 mg/l as 4 mg/l and Mn2+. The obtained fungus body which carried out centrifugal separation was washed by 0.1MEDTA(s)/0.15MNaCl, and was saved -20 degrees C. DNA was extracted according to the general approach. Decomposition of DNA is DNA. It carried out at 50 degrees C for 1 hour using GC analysis kit (YAMASA soy sauce company make). Analysis of a G+C content is detected by 270nm using HPLC (Shimadzu LC-9A and SDPD-6A), a column uses AQ-312 (Product made from Wye ESHII, 6.0x150mm), and it is 10mM. H3 PO4-10mM Elution was carried out by H2 PO4 (pH 3.5**0.01) and flow rate 1.8 ml/min.
[0012] The analysis of 16S ribosomal RNA was adopted as the genetic analysis approach. This analysis used the kit made from PE Applied Biosystems for DNA of the bacillus collected by the approach currently generally performed, and amplified 16SrRNA (s) according to this protocol. In addition, the used primers are 5f, 338f, 515f, 776f, 1087f, 1174f, and 357r, 531r, 810r, 1104r, 1193r and 1540r. The temperature conditions of PCR were performed 30 cycles in 72-degree-C 45 seconds for 60-degree-C 30 seconds for 95-degree-C 30 seconds. Sequence is ABI. PRISM It analyzed using 310. The obtained sequence data are PE Applied Biosystems. The homology to bacillus subtilis was computed using MicroSeq data base of a shrine. Homology was 99.84%. The analyzed base sequences were 1540base(s) from 5base(s), and were the coordination number 1.
[0013] Since many properties of the above-mentioned new microorganism corresponded well with many properties which bacillus subtilis owns, they were named the bacillus subtilis X-2 (Bacillus subtilis X-2). In addition, an applicant for this patent ****s to 1-1-3, **, Higashi, Tsukuba-shi, Ibaraki-ken in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the whereabouts, the Ministry of International Trade and Industry, on September 4, Heisei 12, and the obtained bacillus subtilis X-2 is the trust number FERM. P-18015 is given. This invention also contains that variant and descendant that have the capacity mentioned above as well as this deposition microorganism itself.
[0014]
[Embodiment of the Invention] The new microorganism bacillus subtilis X-2 in this invention is the following, and was made and isolated. It is 102, 104, and 106 with 0.6% brine which sterilized 0.1ml of sludge suspension which distributed the extracted sludge for 10 seconds with the blender. It double-diluted. 0.1ml of each diluent was scattered at the agar flat surface which consists of the below-mentioned A culture medium, and it cultivated at 32 degrees C. Bacterial discernment was based on

microscope observation and biochemical study of the configuration of a colony, and a fungus body. Moreover, since Bacillus **** formed the spore on the flat surface or the slant face, after the colony appeared, the existence of the sporulation of each colony strain was judged two - five days after. When two or more strain was mixed all over the colony, it dissociated with the dilution method further. When a gram negative and Bacillus **** were not able to dissociate with a dilution method, it suspended in 0.6% brine which sterilized the spore of Bacillus **** cultivated and produced on the slant face which consists of the following A culture medium, and isolated except for the gram negative which heats for 10 minutes and cannot be separated 85 degrees C.

A medium composition Neutrient BURORU (Oxoid CM-1) 8g A glucose 8g NaCl 6g Dry-yeast extractives (Difco) 0.2g Agar 14g DW 1000ml [0015] Proteolysis nature was examined about three sorts of isolated strain X-1, X-2, and X-10. The proteolysis trial suspended KUKKUDO meat (NISSUI PHARMACEUTICAL CO., LTD. make and OcidCM-81 are used) 200mg in 6ml of brine 0.5%, 32 degrees C for eight days, measured the suspended solid which carries out shaking culture and remains, and was performed by searching for suspended solid specific consumption by the following formula.

suspended-solid specific-consumption (%) =[(A-B) /A] x100A: The applied KUKKUDO meat suspended solid B: The suspended solid which remained [0016] Although the proteolysis sex-test result of a KUKKUDO meat was shown below, the suspended solid specific consumption of strain X-2 was very high, and it became clear that it was strain with high protease activity. This strain X-2 was named the bacillus subtilis X-2. The bacillus subtilis X-2 had the nucleic-acid base sequence of 16SrRNA(s) of the array number 1.

菌株名	懸濁物質消費率
X－2	８１％
X－１０	１９％
X－I	３％

[Translation done.]